A Pre-Synopsis entitled as

“Control of Stem Rot Disease in Soybean by Using Rhizospheric Bacteria”

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1. Introduction:

Soybean, belonging to the species Glycine max L., happens to be one of the most important oilseed crops grown for its protein-rich seed used in several industrial, food, and agricultural purposes worldwide (Pagano & Miransari, 2016). The Soybean seeds are a key raw material in the protein diet of humans and animals' feed. Its oil is in demand for large quantities in many industrial applications including biofuel, lubricants, and cosmetics in addition to its use as a source of edible oil. According to estimates, global soybean output in 2020-21 is from an area of 136.82 million hectares. It is estimated that World soybean production in 2020-21 will reach 353.47 million tonnes. In the World soybean production scenario, India comes fourth in the produced area with 12.12 million hectares (29.94 million acres) which is 8.86% of the world area and fifth in production with a figure of 11.23 million tonnes in the year 2020-21. Production in Argentina occupies third place in soybean production in the world with 48.80 million tonnes. Brazil has maintained its position as the world's leading soybean producer with an output of 121.80 million tonnes, followed by USA with 112.55 million tonnes and China with 19.60 million tonnes and India with 11.23 million tonnes which account for 34, 32, 14, 6 and 3 percent of world production respectively. The dominant regions for soyabean cultivation in India are Madhya Pradesh, Maharashtra, Rajasthan, Karnataka, and Telangana (Revathi & Reddy, 2022). According to the second advanced estimates 2022-23, Government of India, soybean production as per the first estimate of 2021-22 is pegged at 139.75 lakh tons compared to 129.87 lakh tons. In India Madhya Pradesh is first with 50.18 lakh ha followed by Maharashtra (49.10 lakh ha), Rajasthan (11.51 lakh ha), Karnataka (4.43 lakh ha), Gujarat (2.22 lakh ha) and Telangana (1.75 lakh ha). (https://pjtsau.edu.in/files/AgriMkt/2023/March/Soyabean-March-2023.)

Economically and nutritional speaking, the yield of soybean crop is significantly influenced by several biotic and abiotic stress factors, conditions and methods controlled in which both environmental stresses were reduced. The global soybean production is significantly threatened by soil-borne diseases, which can drastically reduce soybean yields and economic returns. Soil-borne pathogens, including fungi, bacteria, and nematodes, pose a serious challenge to soybean cultivation (Rahman et al., 2024). Among these, collar rot is a particularly devastating disease affecting soybean production. Collar rot disease is also called as stem rot or Southern blight. This destructive disease is caused by the soil-dwelling fungus Sclerotium rolfsii, recognized as one of the most harmful pathogens in agricultural soils. Sclerotium rolfsii has a broad host range, infecting numerous plant species, including peanuts, tomatoes, peppers,

eggplants, and various other vegetables. This soil-borne fungus survives in the soil by forming dark, hard structures known as sclerotia, which enable it to overwinter and persist under unfavourable conditions.

Symptoms of infection include wilting, stem rot, leaf blight, and fruit rot. In warm and humid environments, the disease can spread rapidly, causing significant crop losses. The adaptability of S. rolfsii makes it particularly challenging to eliminate from arable land. It produces a wide array of enzymes capable of degrading plant cell walls and other tissues. Additionally, it secretes secondary metabolites, such as oxalic acid, which are toxic to plant tissues and facilitate its colonization and spread (Paramasivan et al., 2013).

Chemical fungicides have been the ones most used to date against S. rolfsii, however, some disadvantages include environmental harm, non-target effects, and resistance of fungal strains to these fungicides (Abdelaaziz et al., 2024). Other approaches like crop rotation and field cleanliness have also been applied, yet they have proven ineffective in farms with severe infestations. Control practices are now shifting toward eco-friendly and sustainable alternatives, as S. rolfsii shows resistance against conventional methods. Other promising approaches involve biocontrol by rhizospheric bacteria (Jiao et al., 2021).

2. Review of Literature:

Sclerotium rolfsii (teleomorph: Athelia rolfsii (Curzi) Tu and Kimbrough) is a highly destructive soil-borne fungal pathogen that can infect crops at any stage of growth (Punja, 1985). This pathogen has an extensive host range, affecting over 500 plant species worldwide. While it primarily targets dicotyledonous plants, it can also infect a few monocotyledonous species (Hiremath et al., 2024).

2.1 Stem Rot/ collar rot/ Southern blight Disease in Soybean:

Sclerotium rolfsii causes a disease that is seriously threatening soybean cultivation all over the world. According to Chowdary et al., (2024) this disease reduces the quality and yield of crops to a significant level. Symptoms in infected plants include collar rot/stem rot, early wilting, and lesions on the stem. This is mainly a stem-based disease that disrupts the internal transportation of nutrients and water within the plant due to its infection at the base of the stem, hence causing severe stunting, culminating into death (Zakaria, 2023). It includes, among others, soil type, moisture, and temperature. Warm and humid conditions favour its epidemiology; thus, it is of particular importance in tropical and subtropical regions of soybean production. The wide host range further complicates disease management. According to Manasa et al., (2024)

economic damage showed that severe infestation of soybeans may lead to a loss of crop yield of up to 80%. Although resistant soybean cultivars against the disease exist, some agronomic problems in wider application have arisen (Zhu et al., 2022).

2.2 Soybean Stem Rot Disease Management:

Traditionally farmers use synthetic fungicides such as Thiram and Carbendazim (Khodke and Raut (2010), propiconazole 25% EC, thiophanate-methyl 70% WP captan 50% WP, salicylic acid, potassium salt, sorbic acid, carboxin, benomyl, sancozeb, thiovit, dithane M-45, K2HPO4, potassium sorbate, fluazinam, vitavax powder and topsin-M for management of Sclerotium rolfsii Dwivedi and Prasad. (2016). The fungicides play a crucial role in controlling plant diseases. But, excessive application of fungicides causes environmental pollution and has non-target effects on plants and animals. The continuous use of fungicides develops resistance among pathogens, decrease fertility of soil and causes health hazards to animals, human beings and other living organisms (Geiger et al., 2010; Goswami et al., 2018).

Bio management of crop diseases by using healthy rhizospheric microorganism is an innovative approach. This biocontrol approach is environmentally sustainable and minimizes the need for chemical pesticides. Rhizobacteria and fungus besides enhance works growth by elevate nutrient uptake and improving ground health.

Several researchers reported that the rhizospheric microorganisms can be used as biocontrol agent, such as Trichoderma sp. (Priyadharcini et al., 2018; Aryal et al., 2022; Meena et al., 2024), Pseudomonas sp. (Tienda et al., 2020; Dai et al., 2024), Bacillus sp. (Mohammadi et al., 2017; Maksimov et al., 2020; Wang et al., 2020), act as biocontrol agents by promoting plant health through mechanisms like pathogen suppression, nutrient enhancement, and growth promotion. They compete with harmful pathogens for space and nutrients, produce antimicrobial compounds, and trigger plant defines responses. Their use as biocontrol agents offers a sustainable alternative to chemical pesticides.

3. Objectives of the Research Work:

Currently, research all over the world is underway to find and deploy effective biocontrol agents for sustainable agriculture. Sustainable Development Goals elaborated by United Nations target environmentally sound and economically viable farming practices. This not only reduces chemical inputs but also offers a sustainable way of plant disease management. So to attend this disease in ecofriendly cost effective manner, following objectives were set:

1. To isolate the phytopathogen of Stem rot disease (Sclerotium rolfsii) from infected soybean plants and its pathogenicity testing.
2. To isolate the rhizospheric bacteria from healthy crop rhizospheric soil samples
3. To screen rhizospheric isolate for antifungal activity against phytopathogen.
4. Identification of efficient bacterial isolates using morphological, cultural, biochemical and molecular approaches.
5. To reveal the mechanism of microbial control agents
6. To study the growth-promoting ability of microbial control agents
7. To formulate microbial control agents.
8. To perform pot assay for microbial control agents.

4. Material and Methods:

4.1 Isolation of the soybean stem rot pathogens (Sclerotium rolfsii):

The Phytopathogen, Sclerotium rolfsii will be isolated from the infected stems of soybean plants by tissue segment method (Rangaswami and Mahadevan, 1999; Rakh, 2010) on potato dextrose agar (PDA) medium. Soybean plants affected by stem rot disease would be gathered from the Marathwada region and transported to a research laboratory in sterile plastic bags. Plant stems that are infected will be properly cleaned with sterile water. Small pieces of tissue of about 0.5 to 1 cm from infected stem region with some healthy tissues were cut with sterile scalpel. The pieces were surface sterilized with 1% sodium hypochlorite solution for 30 sec. The tissue pieces were subsequently washed in three changes of sterile distilled water to eliminate excess sodium hypochlorite and then the pieces were transferred on to PDA medium in Petri dishes. Plates were incubated at 27±1° C and observed periodically for growth of the fungus. Pure culture of the pathogen was obtained by single hyphal tip method and maintained on PDA.

4.2 Isolation of bacteria from the rhizospheric soil samples:

Soil samples from healthy soybean crop fields from Udgir district were collected in sterile polythene bags and transported to the research lab. One gram of each rhizospheric soil sample will be mixed with 100 ml of sterile distilled water, agitated vigorously for two minutes, and the flask's contents left to remain settled. By using the serial dilution approach, various dilutions of these samples ranging from 10^{-1} to 10^{-6} will be created. For plating, the highest dilutions 10^{-4}, 10^{-5}, and 10^{-6} will be utilized. Pour plate technique was used to plate 0.5 ml of selected dilutions on Nutrient agar medium. Every plate will be incubated at 28 ± 2 °C for 24 to 48 hours (Timonin, 1940).

4.3 Screening for antifungal activity against phytopathogens:

The bacterial isolates will be screened for antifungal activity against phytopathogen (Sclerotium rolfsii) by using a dual culture technique on King B agar plates previously described by Gull and Hafeez (2012).

4.4 Identification efficient of the isolates:

The efficient rhizospheric isolates will be carried out by morphological and biochemical characterization for the primary identification as mentioned in Bergey's Manual of Systematic Bacteriology and molecular approach, i.e. 16S rRNA gene sequencing will opt for the identification up to the species level.

4.5 The biocontrol mechanism of rhizospheric isolates:

The efficient rhizospheric bacterial isolates will be examined for chitinase, volatile metabolites, diffusible metabolite, siderophores etc. in order to determine the mechanism of the microbial control agents.

4.6 Growth promotion ability of efficient microbial Control Agents:

Growth promotion ability of efficient biocontrol agents such as P-solubilization activity will be performed as previously described (Chen et al., 2014) and production of Indole-3-acetic acid (Gordon and Weber, 1952) will be carried out.

4.7 Formulation of microbial control agents:

Bioformulation of microbial biocontrol agents for a sustainable agriculture will be prepared as previously described by Bejarano and Puopolo (2020).

4.7 Pot study of biocontrol agent:

To evaluate potential of efficient microbial control agent in vivo towards Sclerotium rolfsii a Pot Assy experiment will be conducted as previously described by Rakh et al., (2011).

5. Importance of the study:

Microbiological control of soybean stem rot disease by using rhizospheric beneficial microbes like bacteria, fungi, or actinomycetes offer a natural alternative to synthetic chemical fungicides. This approach reducing the need for chemical treatments, improving soil quality and plant health, decrease in production cost, reducing environmental pollution as well as reducing health hazards. These microbes not only control the disease incidence but also promotes the growth of plant by producing some growth hormones.

International status:

The biocontrol of stem rot disease in soybean, primarily caused by Sclerotium rolfsii, has gathered significant international attention due to its eco-friendly and sustainable approach.

Researchers worldwide have explored and developed various biocontrol strategies to manage this devastating pathogen. Below is an overview of the international status of biocontrol efforts:

Oduro et al., (1978) reported that Sclerotium rolfsii a phytopathogen causing Sclerotium rot, Collar and pod rots was observed for the first time on 6 soybean varieties grown at the University of Science and Technology, Kumasi, Ghana. Aken and Dashiell (1991) reported first report of southern blight caused by Sclerotium rolfsii on soybean in Nigeria. Nguyen et al., (2007) reported that soil borne collar rot disease of soybean caused by Sclerotium rolfsii effectively controlled by using bio-gents Trichoderma spp. and Bacillus subtilis.

In a study conducted by Marwan et al., (2017), the antagonistic potential of endophytic bacteria against Sclerotium rolfsii was evaluated using 44 isolates obtained from soybean, rice, and banana. Out of these, 13 isolates were selected in vitro for their ability to control damping-off disease (e.g., BE-CW1, BE-M1, BE-M2, BE-M3, BE-M4, BE-S1, BE-S6, BE-S13, BE-S14, BEBRRG2, BE-KH1), along with fungicides and two controls (healthy and diseased plants). Further evaluation for controlling Sclerotium disease was conducted on six isolates (e.g., BE-M1, BE-M2, BE-M3, BE-M4, BE-CW1, BE-KH2) based on their efficacy against damping-off disease, alongside the same controls. Results indicated that all 13 endophytic bacterial isolates effectively inhibited the mycelial growth and germination of S. rolfsii. Soybean seed treatment by dipping seeds in the bacterial isolates significantly reduced disease incidence and severity. Damping-off disease incidence was reduced by 37.4% to 49.9%, while Sclerotium wilt disease severity decreased by 20.1% to 85%. Additionally, soybean mortality was reduced by 26.3% to 84.4%. Among the isolates, BE-KH2 demonstrated the highest efficacy, suppressing Sclerotium wilt by 85% and reducing soybean mortality by 67.5%.

Five bacterial species were screened by Safni & Antastia (2018), for their antagonistic activity against A. rolfsii using an in vitro dual-culture technique. Among these, Burkholderia cepacia, Stenotrophomonas ficaria, and Vibrio alginolyticus were identified as the most effective antagonists. These species inhibited A. rolfsii with inhibition zone percentages of 98.35%, 97.83%, and 96.97%, respectively. All tested bacterial species demonstrated significant antagonistic activity, with inhibition zone percentages exceeding 60%. In vitro antifungal activity of Trichoderma harzianum against phytopathogen Sclerotium rolfsii causing collar rot disease of soybean using dual culture technique, the percent inhibition of Sclerotium rolfsii induced by T.

harzianum was found upto 78.9% (Hasna et al., 2020).

National status:

In India, stem rot disease caused by Sclerotium rolfsii poses a significant threat to soybean cultivation, leading to considerable yield losses. The growing awareness of environmental sustainability and the harmful effects of chemical fungicides has led to an increasing focus on biocontrol strategies. Below is an overview of the national efforts in managing stem rot disease through biocontrol:

The efficacy of fungal bioagent Trichoderma spp. evaluated against Sclerotium rolfsii. Among the Trichoderma spp. Trichoderma harzianum and Trichoderma viride were most effective in inhibiting the mycelial growth of Sclerotium rolsii by 68 and 67 % respectively (Mandhare and Suryawanshi, 2008). The biological control agents, Pseudomonas fluorescens,

Bacillus subtilis, Pseudomonas sp.-I, Bacillus sp.-I and Bacillus sp.-II were tested in vitro against a phytopathogen Sclerotium rolfsii in dual culture technique inhibited the pathogen up to 76.2, 88.8, 80.01, 35.9and 77.8 % respectively (Vishwanath et al., 2012).

Brunda et al. (2018) investigated the antagonistic activity of 30 soybean endophytes collected from northern Karnataka and parts of Maharashtra against Sclerotium rolfsii, Rhizoctonia bataticola, and Fusarium oxysporum using the dual culture plate technique. Among the tested bacterial endophytes, RB-KK-6 (40.78%), SB-BS-6 (50.08%), and LB-BU-1 (47.02%) demonstrated significant effectiveness in inhibiting the growth of S. rolfsii.

In a study by Hiremath et al., (2024), 75 rhizobacterial isolates, 3 endophytes, and reference cultures were evaluated for their antagonistic activity against Sclerotium rolfsii using the dual culture technique. Two rhizobacterial strains, Streptomyces enissocaesilis (AUUB 209) and Streptomyces racemochromogenes (AUDT 626), showed significant mycelial inhibition, with 76.79% and 64.75% inhibition, respectively. The antagonistic potential of these strains was also attributed to their plant growth-promoting rhizobacteria (PGPR) activities, which include the production of indole acetic acid, siderophores, phosphate solubilization, and chitinase production.

Although this research highlights significant progress in the management of soybean stem rot disease, further studies are necessary to improve the understanding and effectiveness of biocontrol strategies. More research is needed to optimize the use of antagonistic microorganisms, explore new biocontrol agents, and evaluate their long-term performance in diverse environmental conditions to develop sustainable and practical solutions for managing Sclerotium rolfsii in soybean cultivation.

Significance of the study:

Stem rot disease, caused by Sclerotium rolfsii, is one of the most destructive soil-borne diseases affecting soybean crops, leading to significant yield losses worldwide and also in India. As the demand for sustainable agricultural practices increases, the use of chemical fungicides to control this disease has raised concerns due to their environmental impact, potential for resistance development, and harm to non-target organisms. This has highlighted the need for alternative, ecofriendly solutions in the form of biocontrol agents.

Hypothesis:

"Bacteria with bioactive potential obtained from healthy soybean rhizosphere can be used to control the phytopathogens responsible for stem rot disease in soybean plants."

6. Proposed work plan:

Duration		Work Plan
Year I	1-6 months	Course work completion and Review of Literature
	7-12 months	Isolation of the phytopathogens from Stem rot-diseased soybean plants
Year II	1-6 months	Rhizospheric Soil Sample Collection and Isolation of Microorganisms
	7-12 months	Screening of biocontrol agents
Year III	1-6 months	Identification of the isolates
	7-12 months	Production of the bioactive compound and checking its bioactivity
Year IV	1-6 months	Pot study to check the bioactivity of isolates against stem rot disease
	7-12 months	Field study to check bioactivity of isolates against stem rot in soybean crop
Year V	1-6 months	Thesis writing and submission to the University
	7-12 months	Viva voce

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